

WHAT IS CLAIMED IS:

1 1. A method of reassembling polynucleotides involved in
2 transcription, the method comprising,
3 providing a plurality of random polynucleotide segments from one or more
4 transcriptional regulatory progenitor polynucleotides;
5 assembling the plurality of segments in a random fashion, thereby forming
6 a plurality of reassembled polynucleotide; and
7 selecting a reassembled polynucleotide with a different transcriptional
8 regulatory activity than the progenitor polynucleotides.

1 2. The method of claim 1, wherein the segments are from 5 bp to
2 5,000 bp long.

1 3. The method of claim 1, wherein the segments are less than 50 base
2 pairs.

1 4. The method of claim 1, wherein the segments are greater than 49
2 base pairs.

1 5. The method of claim 1, wherein the assembling step comprises
2 ligating the segments.

1 6. The method of claim 5, wherein the ligating step is performed by
2 with a DNA ligase or a topoisomerase.

1 7. The method of claim 1, wherein the plurality of random segments
2 comprises segments from at least two distinct promoter or enhancer polynucleotides.

1 8. The method of claim 1, wherein the plurality of random
2 polynucleotide segments are obtained by random cleavage of one or more transcriptional
3 regulatory progenitor polynucleotides.

1 9. The method of claim 1, wherein the plurality of random
2 polynucleotide segments are obtained by random amplification of one or more part of one
3 or more transcriptional regulatory progenitor polynucleotides.

- 1 10. The method of claim 1, wherein the reassembled polynucleotide
2 comprises a promoter.
- 1 11. The method of claim 1, wherein the reassembled polynucleotide
2 comprises an enhancer.
- 1 12. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with increased transcriptional activity relative to
3 the transcriptional activity of a progenitor polynucleotide.
- 1 13. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with decreased transcriptional activity relative to
3 the transcriptional activity of a progenitor polynucleotide.
- 1 14. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with significant transcriptional activity in at least
3 one cell or tissue type where the progenitor polynucleotide lacks activity.
- 1 15. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide without significant transcriptional activity in at
3 least one cell or tissue type where the progenitor polynucleotide has activity.
- 1 16. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with transcriptional activity that is activated in
3 response to biotic or abiotic stimuli.
- 1 17. The method of claim 1, where the segments are formed by nicking
2 and subsequent end-repair of DNA that is altered by radiation, oxidation, or a chemical
3 agent.
- 1 18. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with transcriptional activity at a different
3 developmental stage of an organism relative to the transcriptional activity of a progenitor
4 polynucleotide.
- 1 19. The method of claim 1, wherein the segments are formed by
2 cleaving one or more progenitor polynucleotides with a restriction endonuclease.

20. The method of claim 1, wherein the segments are formed by cleaving one or more progenitor polynucleotides with DNaseI.

21. The method of claim 1, wherein the segments are formed by cleaving one or more progenitor polynucleotides mechanically.

22. The method of claim 1, wherein the segments are formed in a thermocyclic amplification reaction.

23. The method of claim 22, wherein the thermocyclic reaction is a polymerase chain reaction.

24. The method of claim 23, wherein the polymerase chain reaction is a mutagenic polymerase chain reaction.

25. The method of claim 1, wherein the selection step is performed by ligating the reassembled polynucleotide to a reporter gene and measuring reporter gene activity.

26. The method of claim 1, wherein the plurality of segments further comprises oligonucleotides.

27. The method of claim 26, wherein the oligonucleotide sequence corresponds to a transcription factor binding site.

28. The method of claim 26, wherein the nucleotide sequence of the oligonucleotides are not from a transcriptional regulatory polynucleotide.

29. The method of claim 1, wherein the reassembled polynucleotide is shorter than the progenitor polynucleotide.

30. The method of claim 1, wherein the reassembled polynucleotide is longer than the progenitor polynucleotide.

31. The method of claim 1, wherein the progenitor polynucleotides comprise allelic variants of a transcriptional regulator polynucleotide.

1 32. The method of claim 1, wherein the progenitor polynucleotides
2 comprise plant transcriptional regulatory polynucleotides.

1 33. The method of claim 1, wherein the progenitor polynucleotides
2 comprise yeast transcriptional regulatory polynucleotides.

1 34. The method of claim 1, wherein the progenitor polynucleotides
2 comprise fungal transcriptional regulatory polynucleotides.

1 35. The method of claim 1, wherein the progenitor polynucleotides
2 comprise mammalian transcriptional regulatory polynucleotides.

1 36. The method of claim 1, wherein the progenitor polynucleotides
2 comprise viral transcriptional regulatory polynucleotides.

1 37. The method of claim 1, wherein the progenitor polynucleotides
2 comprise bacterial transcriptional regulatory polynucleotides.

1 38. The method of claim 1, wherein the progenitor polynucleotides
2 consist of one transcriptional regulatory polynucleotide.

1 39. The method of claim 1, wherein the transcriptional regulatory
2 progenitor polynucleotides comprise more than one transcriptional regulatory
3 polynucleotide.

1 40. The method of claim 1, wherein the transcriptional regulatory
2 progenitor polynucleotides are less than 70% identical.

1 41. The method of claim 1, wherein the progenitor polynucleotides are
2 less than 50% identical.

1 42. The method of claim 1, wherein the progenitor polynucleotides do
2 not hybridize to each other following at least one wash in 0.2X SSC at 55° C for 20
3 minutes.

1 43. The method of claim 1, wherein the polynucleotide segments are
2 single stranded.

1 44. The method of claim 1, wherein the polynucleotide segments are
2 double-stranded.

1 45. The method of claim 44, wherein the double-stranded segments
2 have at least one overhanging single-stranded end.

1 46. The method of claim 45, wherein the overhanging single-stranded
2 end comprises fewer than 10 base pairs.

1 47. The method of claim 1, wherein the assembling step does not
2 comprise a polymerase.

1 48. A reassembled polynucleotide of claim 1.